"EVALUATION OF THE EFFICACY OF OZONATED WATER AS AN ADJUNCT TO SCALING AND ROOT PLANING IN THE MANAGEMENT OF CHRONIC PERIODONTITIS: A RANDOMIZED CONTROLLED STUDY"

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In partial fulfilment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In the Specialty of

PERIODONTICS & ORAL IMPLANTOLOGY

Deemed to be University

(Established u/s 3 of the UGC Act, 1956)

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Dr. Tanya Sahai

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORMS
ROS	Reactive Oxygen Species
PI	Plaque Index
PPD	Probing Pocket Depth
CAL	Clinical Attachment Level
BOP	Bleeding on Probing
SRP	Scaling and Root Planing
UNC-15	University of North Carolina-15 Probe
IL-1 β	Interleukin 1-Beta
CP	Chronic Periodontitis

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INTRODUCTION

INTRODUCTION

Inflammation that is host-mediated and microbiologically linked to periodontitis causes the loss of periodontal attachment. Using a standardized periodontal probe to circumferentially check the secondary dentition with respect to cementoenamel junction (CEJ), helps in the identification of clinical attachment loss (CAL)[1]. The intricate interplay between periodonto-pathogenic bacteria and the immune inflammatory retaliation is frequently linked to chronic periodontitis (CP).[2] Alveolar bone loss, clinical attachment loss, gingival inflammation, and periodontal pockets are clinical characteristics of chronic periodontitis that can lead to tooth loss[3]. Two crucial cytokines that initiate and sustain the systemic inflammation are tumor necrosis factor-α (TNFα) and interleukin 6 (IL-6).[4]

The major goal of periodontal therapy is to remove biofilm, stop the disease's progression, and lower inflammation. Reducing etiologic variables below the level that might cause breakdown is the therapy's goal so as to enable the damaged region at a diseased location to be repaired. By using non-surgical periodontal therapy or phase I in conjunction with a chemotherapeutic medication, some techniques might enhance the new attachment of damaged periodontium.[5] Nonetheless, in certain cases, the intricate structure of the root and the lesion's shape may make therapy difficult and prevent the bacterial load from being sufficiently reduced to render the tooth surface physiologically acceptable.[6] This may be connected to the infections' ability to remain in the periodontal pocket during therapy or to the bacteria's generation of particular virulence factors that compromise the host's defenses. It is clear from this that chemotherapeutic agents are highly desirable and might be useful supplements to mechanical treatment.[7]

These supplemental treatments are categorized according to whether they are administered systemically (low concentration at the periodontal pocket) or locally (high concentration at the periodontal pocket) [8]. When compared to systemic treatment, local drug delivery (LDD) for periodontitis reduces the systemic impact by directly delivering an antibacterial agent into subgingival locations. Among the methods are mouth rinses, gels, chips, ointments, and pocket irrigation. Antimicrobials are most often used via subgingival irrigation. Numerous antimicrobial medications, including tetracycline, hydrogen peroxide, boric acid, stannous fluoride, and chlorhexidine, can do this [7.]

Theoretically, therapists utilize chlorhexidine gluconate (CHX), a cationic bis-biguanide salt with a wide antibacterial range, and being regarded as the gold standard, as supra- and sub-gingival irrigant to inhibit bacterial etiologic agents. However, it has been dissuaded as a long-

term therapy option due to some of its adverse effects.[9]

Long-term CHX usage may result in tooth discoloration, mucosal desquamation, and changes in taste perception.[10] Furthermore, the quantity of calculus and stain that form three and six months after SRP when chlorhexidine is administered does not change significantly[11].

Schonbein[12] made the discovery of ozone in 1840, and it has since been employed extensively. He carried out an experiment and discovered that the electrolysis of water resulted in an odor near the positive electrode, which he called ozone.[13] The word "ozein," which means "to have a smell," comes from the ancient Greeks, who also noticed this strong fragrance following electric storms.[13] At ambient temperature, ozone, a triatomic molecule, is an achromatic gas with a strong smell which may be discerned at quantities as low as 0.02 to 0.05 parts per million (by volume)[14]. It's a strong oxidant, very poisonous, and corrosive. The ozone layer, which is located between 10 and 50 kilometers above sea level [9], has the greatest concentrations of ozone in the stratosphere.[15] Since ozone is known to destroy microorganisms by rupturing their cytoplasmic membranes and cell walls. Mono-unsaturated and polyunsaturated fatty acids in the cell wall are chemically altered and fragmented in this process.[16]

Ozone molecules may easily enter cells when the membrane is broken because it becomes more permeable. Because of its antibacterial effect, which comes from oxidizing microbial cellular components and changing subgingival homeostasis, it has lately been recommended as an irrigating agent.[17] Hydroxyl radicals, which are extremely unstable, are produced when ozone dissolves in water. The antibacterial effect is produced by direct interactions of molecular ozone and additional mechanisms that are mediated by free radicals[18]. Studies have demonstrated the antibacterial capabilities of ozonized water against periodontal infections caused by *Aggregatibacter actinomycetemcomitans* and *Porpyromonas gingivalis*.[19,20]

Ozone also has anti-inflammatory, immunostimulation, immunomodulation, biosynthetic, bioenergetic, antihypoxic, analgesic, and hemostatic properties, among other biological effects. Ozone may be utilized in dentistry in a number of ways, including aqueous, gaseous, and oily forms.[21] Fibroblasts, cementoblasts, and epithelial cells are highly biocompatible with aqueous ozone [17,22,23,24.] In aqueous environments, ozone interacts with a wide variety of chemical substances in two different but complementary ways: direct oxidation by the ozone molecule and indirect reactions mediated by hydroxyl radicals. Ozone-induced bacterial death may be influenced by both of these routes.[26]

Numerous writers have argued that subgingival irrigation with ozonated water has greater positive effects than chlorhexidine.[7], [21,25,26]

Ambrosio[27] in his comprehensive systematic review, revealed varying findings on the impact of ozone on periodontal tissue when used in conjunction with scaling and root planing based on clinical and radiographic parameters. However, nothing is known about the biochemical effects of ozone water on chronic periodontitis. Therefore, the investigation aimed to assess the clinical and biochemical effects of ozone water on periodontal tissue by determining how well ozone functions being adjuvant to phase I therapy in patients with chronic periodontitis.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM

To evaluate and compare the effectiveness of scaling and root planing alone with scaling and root planing in conjunction with ozonated water in improving clinical and biochemical markers in individuals with chronic periodontitis**.**

OBJECTIVES

- **PRIMARY OBJECTIVES**

  To evaluate and compare:

  - Periodontal Probing Depth (PPD) within and between the control and test group i.e scaling and root planing (SRP) alone and subgingival irrigation using ozonated water as an adjunct to SRP at baseline, 1 month and 3 months.
  - Clinical Attachment Level (CAL) within and between the two groups at baseline ,1 month and 3 months.
  - Interleukin-1 beta (IL-1β) within and between the control and test group at baseline, 1 month and 3 months.

- **SECONDARY OBJECTIVES**

  To evaluate and compare:

  - Plaque index (PI) within and between the two groups at baseline,1 month and 3 months.

  - Gingival index (GI) within and between the two groups at baseline,1month and 3 months.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Aggarwal P, Bhargava A, Bali S, Sharma VR, Garg A, Mittal N (2024)[28] conducted a study to compare the efficacy of ozonated water and 0.2% chlorhexidine as a subgingival irrigant with scaling and root planing (SRP) in chronic periodontitis. Thirty-one patients with at least one site of 4–6 mm probing pocket depth (PD), bilaterally (either maxillary or mandibular arch) were randomly divided into two groups (Group 1 [0.2% chlorhexidine + SRP], Group 2 [ozonated water + SRP]). Plaque index (PI), gingival index (GI), modified sulcular bleeding index (mSBI), PD, and clinical attachment level (CAL) were recorded at baseline, 1, and 3 months and bacterial count at baseline and 3 months. Paired and unpaired t-test were used for statistical analysis. The authors observed statistically significant reduction in PI, GI, mSBI, probing PD, and CAL in control and test groups at all intervals of the study ($P < 0.05$). The intergroup comparison showed a more statistically significant reduction in the test group versus the control group in PI, GI, mSBI, PD, and CAL and microbial culture at 3 months from baseline. Significant improvement in clinical and microbiological parameters suggests that subgingival ozonated water irrigation can be an effective adjunct to SRP in chronic periodontitis.

Musalaiah S, Kumar PA, Prabhudev PH, Reddy JK, Mandalapu NB, Patcha H (2024)[29] conducted a study to evaluate the efficacy of ozonated water with NSPT compared with scaling and root planing (SRP) alone in treating chronic periodontitis. A total of 30 patients aged 25 to 55 years were divided randomly into two groups having probing pocket depths of ≥ 5 mm. Full mouth SRP was performed in group 1 followed by additional sub-gingival irrigation of ozonated water in group 2. Assessment of plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment levels (CAL) was performed at baseline and six months. Microbiologic assessment with real-time polymerase chain reaction was performed for Dialister pneumosintes and Micromonas micros by collecting plaque samples. The results revealed that there was a reduction in PI, GI, PD, CAL, and microbiologic parameters in both groups following SRP; the results were more significant in group 2 treated by sub-gingival irrigation with ozonated water, when compared with SRP alone in group 1. Also, it was noted that there was a statistically significant reduction of M. micros compared to D. pneumosintes in the ozone-treated group. The subgingival irrigation of ozonated water in conjunction with SRP has beneficial effects in treating patients with chronic periodontitis.

Ambrosio F, Caggiano M, Acerra A, Pisano M, Giordano F (2023)[27] presented a systematic review on whether or not ozone is a valid adjuvant therapy for periodontitis and peri-implantitis. This review was performed in relation to the PRISMA flow chart and was annotated in the PROSPERO register. PICO questions were used as research questions. The risk of bias in the non-randomized clinical trials was appraised using the ROBINS-I tool. In the study a total of 1073 records, in particular, 842 from MEDLINE/PubMed, 13 from Bio Med Central, 160 from Scopus, 1 from the Cochrane library databases, and 57 from the PROSPERO register were evaluated. A total of 17 studies were included in the present systematic review. Information regarding the characteristics of the periodontal, clinical and radiographic parameters for gaseous ozone, ozonate water, ozonate oil, and ozone gel, including clinical attachment loss (CAL) probing depth (PPD), bleeding on probing (BoP), plaque index (PI), gingival index (GI), and marginal bone levels (MBL), were obtained. The studies included in this systematic review show different results regarding the role of ozone in periodontal treatment in association with or without SRP.

Alsakr A, Gufran K, Alqahtani AS, Alasqah M, Alnufaiy B, Alzahrani HG et al (2023)[30]conducted a prospective split -mouth study which aimed to assess the efficacy of ozone therapy in the treatment of stage II and stage III periodontitis. All patients were treated with scaling and root-planing (SRP) on the control side and SRP with ozone therapy on the test side. Probing depth (PD), clinical attachment loss (CAL), O'Leary plaque index (PI), and bleeding on probing (BOP) scores were recorded at baseline and six weeks after the SRP treatment. A total of 46 patients were selected for this study (31 males and 15 females). All periodontal variables (PD, CAL, PI, and BOP) showed significant changes ($p < 0.0001$) from baseline to six weeks in test side as compared to control site.

Ranjith A, Niranjana JM, Baiju KV (2022)[31] conducted a randomized controlled clinical study to evaluate the adjunctive benefit of ozonized water irrigation with mechanical debridement in the management of Stage III periodontitis. The test group (n = 25) was treated with ozonized water irrigation, whereas the control group (n = 25) received normal saline irrigation along with mechanical debridement. Full mouth plaque score, bleeding score, probing pocket depth and clinical attachment loss were evaluated at baseline and 4 weeks after treatment. Salivary interleukin 1 beta was analyzed using enzyme linked immunosorbent assay. Analysis of covariance, t test and chi-square test were used for intergroup comparison. Intragroup comparison was done using the paired t test. Adjunctive ozone water irrigation

resulted in significant improvement in all clinical parameters, except probing pocket depth after adjusting the extraneous effects due to initial confounding factors ($p < 0.001$). But as per subgroup analysis, ozone water irrigation resulted in significant reduction in pocket depth in deep pockets ($p = 0.01$) and the number of sites with a pocket depth ≥4 mm with bleeding on probing. Salivary interleukin 1 beta also reduced significantly in the test group after therapy. The authors concluded that ozone irrigation provides adjunctive benefit along with nonsurgical periodontal therapy in reducing clinical parameters and inflammatory mediators in saliva. But long-term benefits need to be assessed with future studies.

Ramirez-Peña AM, Sánchez-Pérez A, Campos-Aranda M, Hidalgo-Tallón FJ (2022)[32] conducted a study to assess the effectiveness of ozone therapy as an adjunct to mechanical therapy in periodontitis patients. Thirty-two patients diagnosed with generalized periodontitis were selected, with a total of 655 teeth examined. Each patient's mouth was divided into four quadrants (the split-mouth model) to be randomly treated with four sessions of gaseous ozone or air. Gingival index, clinical attachment loss, miller's mobility index and the clinical improvements, were assessed through the visual analog scale (VAS). In addition, the microorganisms were qualitatively compared. After four weeks of treatment, the teeth of the ozone-treated quadrants showed statistically significant reductions in the gingival index and an improvement in the clinical attachment ($p < 0.0001$). The same treatment also significantly improved mobility by between 70% and 86% compared to the control group ($p < 0.0001$). Statistically significant differences were also recorded for the VAS ($p < 0.0001$). In the qualitative study of the subgingival flora, significant differences were observed ($p < 0.0001$). The overall results of this trial support the view that ozone treatment is effective and well tolerated in cases of generalized chronic periodontitis.

Sharma P, Khiraiya N, Kareem G, Raj S, Agrawal A, Bhatnagar TK, et al (2022)[33] conducted a study on 60 patients with 186 sites with PPD >5 mm, PI >2, GI >2. Scaling and root planing was performed meticulously and on the same day ozonated olive oil irrigation was performed and the healing of gingiva was checked by clinical parameters after 1 month. Statistically significant reduction in PPD, plaque index was seen at the end of 1 month. Use of ozonated olive oil irrigation as an adjunct to scaling and root planing showed good results in reduction of clinical parameters, in patients with chronic periodontitis.

Deepthi R, Bilichodmath S (2020)[21] conducted a metanalysis to review literature on the

effects of ozone as an adjunct to mechanical therapy in periodontitis patients. A search of literature was conducted to identify articles of ozone therapy in periodontitis published during the period from January 1, 2010, to July 30, 2018. PubMed, Medline, Embase, Cochrane, and Google Scholar search and hand searching of journals were conducted to identify relevant articles. Out of a total of 123 studies that fit the initial inclusion criteria, 117 studies were further excluded. Only six studies were included in the meta-analyses. A high level of heterogeneity in the selected studies was found as demonstrated by Q-value of 10.241 and I^2 value of 80.49%. However, the funnel plots showed symmetrical shape, with prevalence studies indicating absence of publication bias. Authors concluded that ozone therapy can be used effectively as an adjunct to scaling and root planing in the treatment of periodontitis.

Moreo G, Mucchi D, Carinci F (2020)[34] conducted a randomized clinical trial to check efficacy of ozone therapy in reducing oral infection of periodontal disease. Inclusion Criteria were 5 men + women each with age range of 55 ± 7 years with a diagnosis of chronic periodontitis. None of these patients received any surgical or non-surgical periodontal therapy and demonstrated radiographic evidence of moderate bone loss. The upper and lower right quadrants were treated with ultrasonic scaler, the left quadrants with ultrasonic scaler with ozonated water. Ten microbiological samples were collected from upper left quadrants and 10 from upper right quadrants from each patient. Microbiological samples were collected from the sites of the patients at baseline and at the 7th day. After the treatment with ozonized water, a remarkable decrease in bacteria amount, both for some species and for the total count was observed in the left quadrants respect to right ones. The study demonstrated the efficacy of the ozonized water in the management of moderate to severe chronic periodontitis

Vasthavi C, Babu HM, Rangaraju VM, Dasappa S, Jagadish L, Shivamurthy R (2020)[35] conducted a randomized clinico-microbial study to evaluate ozone as an adjunct to scaling and root planing in the treatment of chronic periodontitis. Twenty-four patients with chronic periodontitis selected for the study were randomly divided into Group A and Group B, receiving ozone water irrigation and distilled water irrigation, respectively, after scaling and root planing. Subgingival plaque was collected from the selected investigational teeth and was analyzed using BANA-Zyme™ Processor to evaluate the "red complex" periodontal pathogens. The clinical and microbiological parameters were recorded at baseline, 14 days, 21 days, and 2 months. The mean probing pocket depth scores for Group A and Group B at the baseline were 6.833 ± 1.193 and 7.833 ± 1.276; on day 14th, they were 6.616 ± 1.403 and 7.083

± 1.378; on day 21st, they were 5.166 ± 0.937 and 6.083 ± 1.443; and on the 2nd month, they were 4.500 ± 0.797 and 5.166 ± 1.029, respectively. At the 2nd month, in Group A, 9 samples showed BANA negative and 3 samples showed BANA positive, and in Group B, 12 samples showed BANA negative and 0 sample showed BANA positive. The microbiological analysis showed a reduction in periodontal pathogens in both the groups. Significant improvement in both clinical and microbiological parameters suggests that subgingival ozonated water irrigation could be an efficient adjunct to scaling and root planing in the treatment of chronic periodontitis.

Khare M, Jain KS, Sharma N (2019)[7] conducted a study to evaluate the effect of using ozonated olive oil along with SRP, compared to subgingival chlorhexidine with SRP for treatment of chronic periodontitis. In this split-mouth design, twenty untreated chronic periodontitis patients who met the criteria of having periodontal pocket with depth ≥4mm and bleeding on probing (BOP) in at least two different quadrants were included. Group 1 (control) subgingival chlorhexidine application was done and in group 2 (test) ozonated olive oil application with SRP was done. Clinical parameters were evaluated at baseline, 7 days, 14 days and 21 days post treatment and plaque samples at baseline and 21 days. Microbiological analysis was done to assess the change in level of P. gingivalis. Both the groups (test & control) showed improvement in all the recorded parameters (PI, GI, PPD& microbiological analysis) while the test group showed statistically significant reduction in all the clinical parameters compared to control group ($p < 0.05$) except the microbiological analysis. Authors concluded that a single subgingival irrigation with ozonated oil can be effectively used as an adjunct to SRP in non-surgical management of chronic periodontitis.

Kaur A, Bhavi K, Das SK, Khanna SS, Jain S, Kaur A (2019)[36] conducted a study to evaluate the efficacy of irrigation of periodontal pockets by using ozonated water and 0.2% chlorhexidine (CHX) gluconate as adjuncts to scaling and root planing in the management of chronic periodontitis.20 patients in the age group of 30–60 years, suffering from chronic periodontitis presenting with at least one site with an almost similar pocket depth of 4–6 mm in both the quadrants of maxillary arch was taken. Irrigation was done after 2 weeks of scaling and root planing on the same day with ozonated water and 0.2% chlorhexidine gluconate for two and half minutes. The clinical parameters like gingival index, probing pocket depth (PPD) and clinical attachment level (CAL) were recorded for both the groups at the baseline visit. They were subsequently recalled after 4 weeks and 3 months interval from the baseline visit.

The authors concluded that subgingival irrigation with ozonized water is beneficial than present conventional therapeutic modalities. Ozonated water restricts the formation of dental plaque and reduces the number of subgingival pathogens thereby treating periodontal diseases.

Seydanur DE, Serkan D, Abubekir E, Aysun BK, Onder O, Arife C et al (2019)[37] conducted a study to evaluate the clinical and biochemical (oxidative stress and pro-inflammatory mediators) effects of the gaseous ozone use accompanied by scaling and root planning (SRP) in periodontal treatment. The study population consisted of 40 patients with chronic periodontitis (CP) randomly sorted into two groups of 20. The experimental group received SRP plus 3 watts gaseous ozone in two separate applications five days apart, whereas the control group received SRP plus placebo. Clinical periodontal parameters were assayed and saliva samples were taken before the initial and one month after the second treatment. Periodontal examination assessed plaque index (PI), gingival index (GI), probing depth, and clinical attachment level (CAL). Total antioxidant status (TAS), total oxidant status (TOS), nitric oxide (NO), 8-hydroxy-2'-deoxyguanosine (8-OHdG), myeloperoxidase (MPO), glutathione (GSH), malondialdehyde (MDA), and transforming growth factor-beta (TGF-β) levels were evaluated from saliva samples. Changes following treatment in PI, GI, probing depth, and CAL scores were similar for both groups ($p>0.05$). Of note, TGF-β levels were observed to be higher in the treatment group than in controls ($p<0.05$). Changes in 8-OHdG, TAS, TOS, NO, MPO, GSH and MDA levels, however, were not significantly different between groups ($p>0.05$).

Uraz A, Karaduman B, Isler SÇ, Gönen S, Çetiner D (2018)[38] conducted a study to evaluate the clinical, biochemical and microbiological efficacy of ozone treatment as an adjunct to scaling and root planing (SRP) in generalized chronic periodontitis (GCP) patients. Eighteen patients (9 males and 9 females; aged from 28 to 47 years, mean age of 40 ± 6.51 years) with GCP were recruited in the study. In a split mouth design, two quadrants in each patient were randomly allocated to SRP-alone or SRP-ozone therapy (SRP + OT) groups by coin toss method. Subgingival plaque and gingival crevicular fluid (GCF) samples were collected at baseline, following 1st and 3rd months. The clinical parameters were monitored at baseline and after 3 months. Microbiological parameters were analysed by quantitative-PCR and GCF biomarkers were determined by ELISA. Results were analysed statistically. Statistically significant improvements in all clinical parameters were accompanied by a reduction in microbiological and biochemical parameters in both treatment groups. SRP treatment resulted

in a significant reduction of *Porphyromonas gingivalis* (*Pg*) at 1st month and *Tannerella forsythia* (*Tf*) and *Prevotella intermedia* (*Pi*) at 3 months. Following SRP treatment, the interleukin (IL)-8 levels were significantly reduced at month 1. There were no significant differences. Within the limitations of this study, adjunctive ozone therapy did not provide additional benefits to clinical, microbiological and biochemical parameters over SRP in chronic periodontitis patients.

Paniz G, Nart J, Gobbato L, Chierico A, Lops D, Michalakis K (2016)[39] conducted a prospective randomized clinical study on 113 patients with periodontal disease. Local oxygen–ozone therapy was used in combination with traditional mechanical therapy versus the use of mechanical therapy alone in a group of patients with periodontal disease. After 6 months of root planing, the individuals who received oxygen–ozone therapy as an integral part of the periodontal treatment had significant stabilization of the clinical values. It was observed in particular that in both groups that the deepest pockets (PD > 6 mm) had the greatest decrease.

Issac AV, Mathew JJ, Ambooken M, Kachappilly AJ, Pk A, Johny T et al (2015)[40] conducted a study to evaluate the effect of ozonized water on microbiologic parameters and clinical parameters namely gingival index, probing pocket depth, and clinical attachment level at baseline and 4 weeks. Thirty chronic periodontitis patients with probing pocket depth ≥6mm on at least one tooth on contra-lateral sides of opposite arches were included in the study. The test sites were subjected to ozonized water subgingival irrigation with subgingival irrigation device fitted with a modified subgingival tip. Control sites were subjected to scaling and root planing only. Microbiologic sampling was done in the control and test group at baseline and at 4 weeks. The results were statistically analysed using independent t-test and paired t-test. The results showed that ozonized water subgingival irrigation can improve the clinical and microbiologial parameters in patients with chronic periodontitis when used as an adjunct to scaling and root planing.

Habashneh AL, Alsalman W, Khader (2014)[41] conducted a study was to determine the clinical and biological effects of the adjunctive use of ozone in nonsurgical periodontal treatment. Forty-one patients with chronic periodontitis were randomized to treatment with either subgingival scaling and root planing (SRP) followed by irrigation with ozonated water (test) or subgingival SRP followed by irrigation with distilled water irrigation (control). Plaque index; gingival index; bleeding on probing; probing pocket depth; gingival recession; and clinical attachment loss, C-reactive protein was measured at baseline and three months. The

study showed that there was no significant difference in any study parameter between test and control groups. Hence the authors concluded that irrigation with ozonated water as an adjunctive therapy to SRP produces no statistically significant benefit compared with SRP plus distilled water irrigation

Yılmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T (2013)[42] conducted a study to evaluate the clinical and microbiological results of treatment with Er: yttrium aluminum garnet (YAG) laser and topical gaseous ozone application as adjuncts to initial periodontal therapy in CP patients. Thirty patients with CP were randomly divided into three parallel groups, each composed of ten individuals. Group 1: SRP + Er: YAG laser; Group 2: SRP + topical gaseous ozone; and Group 3: SRP alone. The clinical parameters were monitored at day 0 and day 90, such as PI, SBI, PD, and relative attachment levels were measured. Statistically significant improvements in clinical parameters were observed within each group.

Katti SS, Chava VK (2013)[43] conducted a study to evaluate the clinical effects of ozonated water on periodontal tissues. In this study 30 subjects were selected with age ranging from 20 to 60 years and pocket depth of ≥5mm. Two sites were selected in each patient which were divided into two groups, group 1(control group-irrigation with saline) and group 2(study group-irrigation with ozonized water) and clinical parameters were recorded at baseline, 15 days and 30 days. When the comparison of mean values of plaque index and gingival index between the groups and at different time intervals were made, statistically significant difference was observed at 30 days at 5% level. When the mean values of clinical attachment level on mesial and distal site were compared between the groups, statistical significance was observed at 5% level and 1% level respectively. Similarly, statistical significance at 5% level was observed at 15 and 30 days on buccal site. Subgingival irrigation with ozonized water is beneficial adjunct treatment modality to enhance periodontal health with significant role in periodontal therapy.

Iliadis D, Millar B (2013)[44] conducted a study to evaluate the effects of ozone in periodontal treatment in dental practice. Twenty-five patients were treated with gaseous ozone after having had failed conventional periodontal treatment. BPE scores and the six deepest pockets were measured in each patient before and after the use of ozone. Twenty-five patients attended both follow up appointments. Based on BPE scores, twenty of the patients had overall improvement while five of the patients continued to have deterioration. Eight patients had an improvement in depths of periodontal pockets by three millimeters, sixteen patients had improvement by

one to two millimeters and one patient did not improve. The depth of pockets after the use of ozone decreased significantly ($P < 0.001$). The study revealed that gaseous ozone significantly ($P < 0.001$) reduced the depth of pockets in patients with periodontal disease. The positive results encourage further investigation in the subject.

Hayakumo S, Arakawa S, Mano Y, Izumi Y (2013)[45] conducted a study to evaluate the clinical and microbiological effects of ozone nano-bubble water as an adjunct to scaling and root planing. Ozone nano-bubble water (NBW3) seems to be suitable as an adjunct to periodontal treatment owing to its potent antimicrobial effects, high level of safety, and long storage stability. Twenty-two subjects were randomly assigned to one of the two treatment groups: full-mouth mechanical debridement with tap water or full-mouth mechanical debridement with NBW3 (NBW3). Clinical examination was performed at baseline and 4 and 8 weeks after treatment. Microbiological examination was carried out before and after treatment and at 1- and 8-weeks posttreatment. There were significant improvements in all clinical parameters after 4 weeks in both groups. The reduction in the probing pocket depth and the clinical attachment level gain after 4 and 8 weeks in the NBW3 group were significantly greater than those in the WATER group. Moreover, only the NBW3 group showed statistically significant reductions in the mean total number of bacteria in subgingival plaque over the study period. The authors suggested that subgingival irrigation with NBW3 may be a valuable adjunct to periodontal treatment.

Kshitish D, Laxman VK (2010)[26] conducted a clinical and microbiologic study to check the use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients. Subgingival plaque is associated with Porphyromonas species and Actinobacillus actinomycetemcomitans (Aa), in the subgingival environment. Apart from bacteria, certain viruses and fungi that are associated with periodontal disease are also present in the subgingival plaque. A total of 16 patients suffering from generalized chronic periodontitis were selected for the study. The study period of 18 days was divided into two time-intervals, i.e. baseline (0 days) to 7th day, with a washout period of 4 days followed by a second time interval of 7 days. The use of ozone and chlorhexidine gluconate (CHX) irrigation was randomized. A higher percentage of plaque index (12%), gingival index (29%) and bleeding index (26%) reduction was observed using ozone irrigation as compared to chlorhexidine. The percentile reduction of Aa (25%) using ozone was appreciable as compared to no change in Aa occurrence using chlorhexidine. By using O3 and chlorhexidine, there was no antibacterial effect on

Porphyromonas gingivalis (Pg) and Tannerella forsythensis. The antifungal effect of ozone from baseline (37%) to 7 the day (12.5%) was pronounced during the study period, unlike CHX, which did not demonstrate any antifungal effect. Authors concluded that ozone may be considered as an alternative management strategy due to its powerful ability to inactivate microorganisms.

Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R et al (2006)[22] conducted a study to investigate whether gaseous ozone (4.106 l/gm) and aqueous ozone (1.2520 l/gm) exert any cytotoxic effects on human oral epithelial (BHY) cells and gingival fibroblast (HGF-1) cells compared with established antiseptics [chlorhexidine digluconate (CHX) 2%, 0.2%; sodium hypochlorite (NaOCl) 5.25%, 2.25%; hydrogen peroxide (H2O2) 3%], over a time of 1 min, and compared with the antibiotic, metronidazole, over 24 h. Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated. Ozone gas was found to have toxic effects on both cell types. Essentially no cytotoxic signs were observed for aqueous ozone. CHX (2%, 0.2%) was highly toxic to BHY cells, and slightly (2%) and non-toxic (0.2%) to HGF 1 cells. NaOCl and H2O2 resulted in markedly reduced cell viability (BHY, HGF-1), whereas metronidazole displayed mild toxicity only to BHY cells. Taken together, aqueous ozone revealed the highest level of biocompatibility of the tested antiseptics.

Ramzy M, Gomaa HE, Mostafa MI, & Zaki BM (2005)[46] irrigated the periodontal pockets with 150mL of ozonized water over 5-10 min once weekly for a clinical 4week study using a blunt tipped sterile plastic syringe in 22 patients suffering from aggressive periodontitis. Highly significant improvement regarding pocket depth, plaque index, gingival index and bacterial count was recorded related to quadrants treated by scaling and root planing together with ozone application. Patients were clinically and radiographically examined. Scaling, root planing, oral hygiene measures with or without ozone application were followed. Different treatment plans were evaluated regarding plaque and gingival indices, pocket depth, attachment loss and mobility scores. Antibacterial effect of ozone was investigated. Authors also reported significant reduction in bacterial count in sites treated with ozonized water and recommended the use of oral hygiene instructions, scaling and root planing together with subgingival irrigation with ozonized water in the management of aggressive periodontitis.

Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA (2005)[47] evaluated the potential of ozone as an anti-bacterial agent using Enterococcus faecalis as a test species. Ozone was used

in both gaseous (produced by Purezone device), and aqueous forms (optimal concentrations 0.68 mg/l). It was concluded that ozone in solutions was antibacterial against planktonic Enterococcus faecalis after 240s treatment. However, it was not effective against Enterococcus faecalis cells in a biofilm unless they were displaced into the surrounding medium by agitation. Gaseous ozone was not effective on the Enterococcus faecalis biofilm. The antibacterial efficacy of ozone was tested against both broth and biofilm cultures. Ozone was sparged for 30, 60, 120 and 240 s, through overnight broth cultures of a strain of E. faecalis (E78.2) and compared with those that were centrifuged, washed and re-suspended in water. Enterococcus faecalis (E78.2) biofilms were grown on cellulose nitrate membrane filters for 48 h and suspended in water through which ozone gas was sparged with stirring for 60, 120 and 240 s in a standard fashion. In a separate test, biofilms were also exposed to gaseous ozone. Sodium hypochlorite (NaOCl) was used as a positive control. There were significant ($P < 0.05$) reductions of bacteria in the unwashed (2 log (10) reductions) and washed (5 log (10) reductions) broth cultures following 240 s applications. Biofilms incubated for 240 s with ozonated water showed no significant reduction in cell viability attributable to ozone alone, whereas with NaOCl no viable cells were detected over the same time. Gaseous ozone applied for 300 s had no effect on these biofilms. Ozone had an antibacterial effect on planktonic E. faecalis cells and those suspended in fluid, but little effect when embedded in biofilms. Its antibacterial efficacy was not comparable with that of NaOCl under the test conditions used.

Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T (2004)[17] conducted a study to evaluate effect of ozonated water on oral microorganisms and dental plaque. Almost no microorganisms were detected after being treated with ozonated water (4 mg/l) for 10 s. To estimate the ozonated water-treated Streptococcus mutans, bacterial cells were stained with live/dead. BacLightTM Bacterial Viability Kit. Fluorescence microscopic analysis revealed that S. mutans cells were killed instantaneously in ozonated water. Some breakage of ozonated water-treated S. mutans was found by electron microscopy. When the experimental dental plaque was exposed to ozonated water, the number of viable S. mutans remarkably decreased. Ozonated water strongly inhibited the accumulation of experimental dental plaque in vitro. After the dental plaque samples from human subjects were exposed to ozonated water in vitro, almost no viable bacterial cells were detected. These results suggested that ozonated water should be useful in reducing the infections caused by oral microorganisms in dental plaque.

Thanomsub B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C (2002)[48]conducted a study which showed that ozone appeared to inhibit growth and caused the death of gram negative and gram-positive tested bacteria: Escherichia coli, Salmonella sp, Staphylococcus aureus and Bacillus subtilis. Bacterial cultures at 10(3), 10(4), 10(5), 10(6), and 10(7) cfu/ml dilution were exposed to 0.167/mg/min/L of ozone at different time intervals (0, 5, 10, 15, 30, 60, 90, 120, and 150 min). Cell viability was observed in all types of tested bacteria at 10(3), 10(4), 10(3) cfu/ml within 30 min after ozone exposure. However, cell inactivation was not significantly observed at concentrations of 10(6), 10(7) cfu/ml even after an exposure of 150 min. Ultrastructural changes of treated bacteria showed deformation, rough damage and surface destruction revealed by scanning electron microscopy. Some bacterial cells showed collapsed and shrunken patterns within 60 min and severe rupture and cellular lysis after 90 min of ozone treatment. This study supports the proposed mechanism of the bacteria inactivation by ozone that caused cell membrane destruction and finally lysis reaction. Thus, the precaution of using ozone as a biocide should be used to address appropriate concentrations of bacterial contamination in water.

MATERIALS AND METHODOLOGY

MATERIALS AND METHODOLOGY

SOURCE OF DATA

Amongst the patient reporting to the Out Patient Department of the Department of Periodontics & Oral Implantology, Santosh Dental College, Ghaziabad, 72 patients were selected for proposed trial meeting the inclusion and exclusion criteria. and written consent was obtained prior to the procedure. The study was accepted by institutional ethical committee.

CRITERIA FOR SELECTION OF SAMPLE

- Patients with Probing Pocket depth of 4-6mm
- Sites selected in each patient was randomly divided into 2 Groups using coin toss method:

- Group A (Control) -scaling and root planing
- Group B (Test) - scaling and root planing with ozonated water irrigation(subgingival)

INCLUSION CRITERIA –

- Systemically healthy patients in the age group of 30-60 years
- Probing Pocket Depth of 4-6mm
- Has not received any surgical or non-surgical periodontal therapy in past 6 months.

EXCLUSION CRITERIA –

- Antimicrobial medications or antibiotic mouth rinses taken in past 6 months prior to study.
- Medication that would induce gingival enlargement, immunosuppressants
- Pregnant women/Lactating mothers.
- Smokers and tobacco chewers.

STUDY DESIGN

In this randomized controlled trial patients were selected according to inclusion and exclusion criteria. On the day of treatment, a detailed case history was recorded and complete patient examination was done.

Clinical and biochemical parameters such as probing pocket depth (PPD), clinical attachment level (CAL), salivary interleukin-1 beta (IL-1β), plaque index (PI) and gingival index (GI) were recorded preoperatively (baseline).

Patients were segregated into two groups:

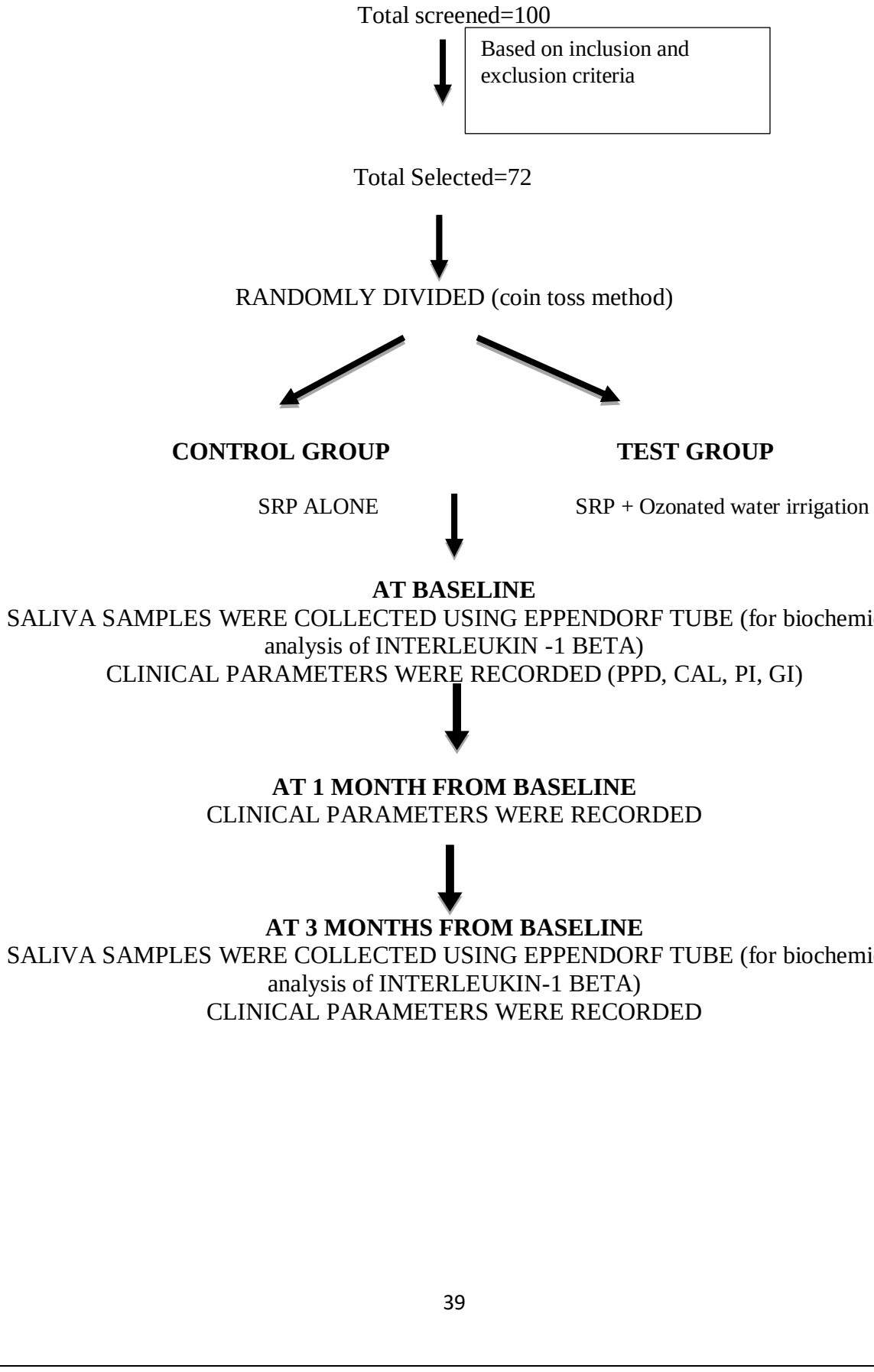
GROUP A (CONTROL): - scaling and root planing done.

GROUP B (TEST): - scaling and root planing along with ozonated water irrigation done.

After proper scaling and root planing was done, saliva samples were collected in the Eppendorf tubes. Saliva samples were then placed in an ice box and then transported to the lab. Subgingival irrigation with 2mL of ozonated water using 3mL syringe was done for the patients in Group B.

Patients were recalled after 1 and 3 months for assessment of clinical and biochemical parameters.

STUDY DESIGN


Total screened=100
Based on inclusion and exclusion criteria
Total Selected=72
RANDOMLY DIVIDED (coin toss method)
CONTROL GROUP
TEST GROUP
SRP ALONE
SRP + Ozonated water irrigation
AT BASELINE
SALIVA SAMPLES WERE COLLECTED USING EPPENDORF TUBE (for biochemical analysis of INTERLEUKIN -1 BETA)
CLINICAL PARAMETERS WERE RECORDED (PPD, CAL, PI, GI)
AT 1 MONTH FROM BASELINE
CLINICAL PARAMETERS WERE RECORDED
AT 3 MONTHS FROM BASELINE
SALIVA SAMPLES WERE COLLECTED USING EPPENDORF TUBE (for biochemical analysis of INTERLEUKIN-1 BETA)
CLINICAL PARAMETERS WERE RECORDED

CLINICAL PARAMETERS INCLUDED

1. PROBING POCKET DEPTH

- measured from the free gingival margin to the most apical part of the sulcus, using a UNC-15 probe.

2. CLINICAL ATTACHMENT LEVEL

- calculated by measuring the distance between cementoenamel junction and the most apical part of the sulcus with the help of UNC-15 probe.

3. PLAQUE INDEX [49] *(Silness P and Loe H, 1964)*

-measures the thickness of plaque on the tooth.

Scoring system- Scoring was done on Index teeth (teeth with PPD=4-6mm). The surfaces examined on each tooth are distofacial, facial, mesiofacial and lingual.

SCORING CRITERIA

0-No plaque.

1-A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using disclosing solutions or by using probe.

2-Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/ or adjacent tooth surface, which can be seen with naked eye.

3-Abundance of soft matter within the gingival pocket and/ or on gingival margin and adjacent tooth surface.

CALCULATION

The scores from four areas of the tooth are added and divided by four

INTERPRETATION

Excellent	0
Good	0.1-0.9
Fair	1.0-1.9
Poor	2.0-3.0

4.GINGIVAL INDEX[50] (Loe H and Silness P, 1963)

- to assess the clinical severity of gingivitis (Gingival Inflammation).

Scoring system- Scoring was done on Index teeth (teeth with PPD=4-6mm). The surfaces examined on each tooth are distofacial facial, mesiofacial and lingual.

SCORING CRITERIA

0-Healthy Gingiva (no change in color of gingiva, no inflammation, no bleeding on probing)

1-Mild Inflammation (slight change in color or gingiva, mild edema, no bleeding on probing)

2-Moderate Inflammation (Redness, hypertrophy, edema and glazing, bleeding on probing)

3-Severe Inflammation (marked redness, hypertrophy, edema and ulceration spontaneous bleeding on probing)

CALCULATION

The scores from four areas of the tooth are added and divided by four

INTERPRETATION

GINGIVAL SCORE	**DEGREE OF GINGIVITIS**
0.1-1.0	MILD
1.1-2.0	MODERATE
2.1-3.0	SEVERE

ARMAMENTARIUM:

- Mouth mirror
- Explorer
- UNC-15 probe
- Pair of tweezers
- Gloves
- Mouth masks
- Head Cap
- Cotton
- Ultrasonic scaler
- Eppendorf tubes
- Ozonated water
- Kidney trays
- Disposable syringe
- Elisa kit (IL-1β) (Elabscience™)

COLOR PLATES

Figure 1: Clinical Armamentarium air-based ozone generator

Figure 2: Biochemical armamentarium Eppendorf tubes for saliva sample collection

Figure 3: Transport armamentarium Ice box

CONTROL GROUP

gure 4: Probing pocket pth at baseline

Figure 5: Scaling and Root Planing

Figure 6: Probing pocket depth at 1 month post SRP

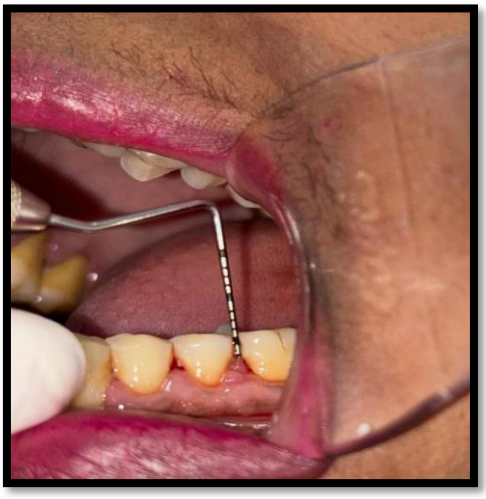


Figure 7: Probing pocket depth at 3 months post SRP

Figure 8: Clinical Attachment level at baseline

Figure 9: Clinical Attachment level at 1 month post SRP

Figure 10: Clinical Attachment level at 3month post SRP

TEST GROUP

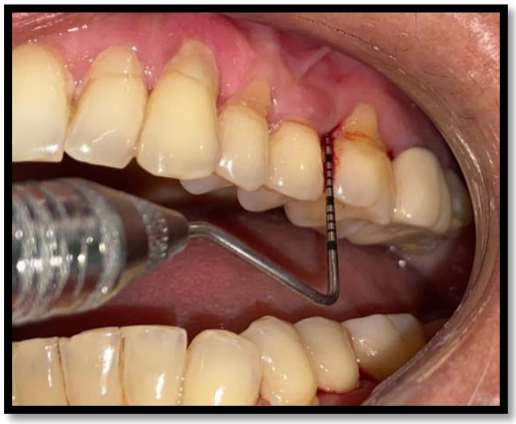


Figure 11: Probing pocket depth at baseline

Figure 12: Ozone water irrigation

Figure 13: Probing pocket depth at 1 month post irrigation

Figure 14: Probing pocket depth at 3-month post irrigation

Figure 15: Clinical attachment level at baseline

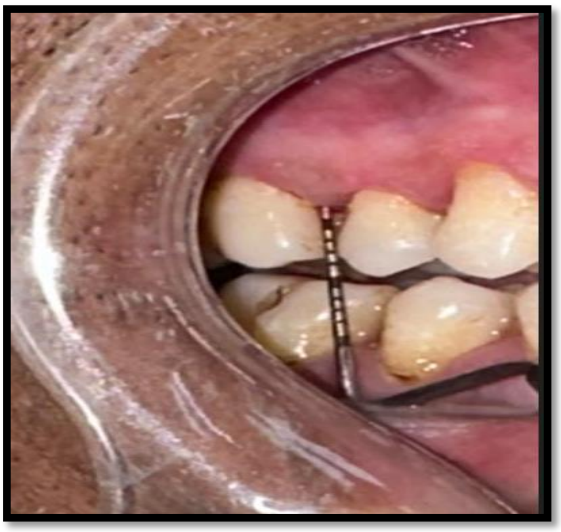


Figure 16: Clinical attachment level at 1 month post irrigation

Figure 17: Clinical attachment level at 3month post irrigation

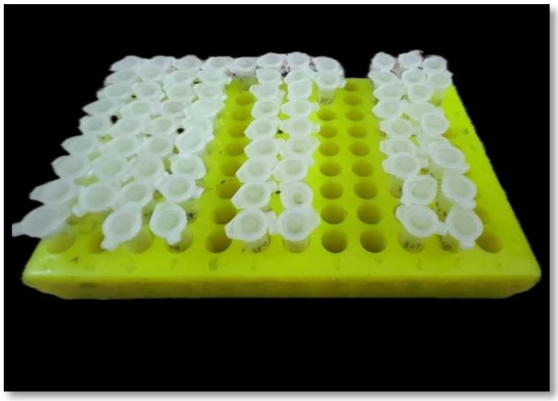


Figure 18: Saliva Sample in Eppendorf Tubes

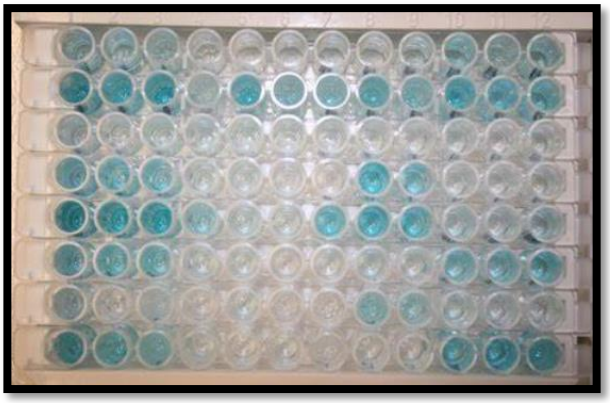


Figure 19: ELISA well plate with saliva samples

Caution
www.elabscience.com
If use the kit within one month, store at 4°C.
Otherwise, take out Micro ELISA plate, Reference Standard, Concentrated Biotinylated Detection Ab/Ag and Concentrated HRP Conjugate to store at -20 °C. Keep other reagents at 4 °C.
Elabscience®
Enzyme Linked Immunosorbent Assay Kit
Human IL-1β(Interleukin 1 Beta) ELISA Kit

Figure 20: ELISA Kit (Elabscience™)

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Descriptive statistics was performed by calculating mean and standard deviation for the continuous variables. Categorical variables are presented as absolute numbers and percentage. The data was entered into Microsoft Excel and analyzed using SPSS (Statistical Package for Social Sciences) package 26.0 for relevant statistical comparisons. Results presented in the form of tables and graphs.

Shapiro Wilk test was used to check whether the continuous variables were following normal distribution or not. Student independent sample t-test was used for the comparison of values between two groups while intragroup comparison was done using paired t-test between 2-time intervals. Level of statistical significance was set at p-value less than or equal to 0.05.

The statistical tests used were:

- **Unpaired or Independent t-test** is used for comparison of mean value between 2 groups when the data follows normal distribution.
- **Paired or Dependent t-test** was used for comparison of 2 mean values obtained from a same group or a pair of values obtained from the same sample when the data follows normal distribution.
- The **p-value** was taken significant when less than 0.05 **($p<0.05$)** and confidence interval of 95% was taken.

The following formulas were employed for calculation for various parameters:

1. **Mean/ Average**

Mean or Average is defined as the sum of all the given elements divided by the total number of elements.

Mean = sum of elements / number of elements.

It is denoted by the letter X.

$$X = \frac{\sum X}{\text{No. of observations (n)}}$$

2. Standard Deviation

The **standard deviation** of a statistical population, a data set, or a probability distribution is the square root of its variance. Standard deviation is a widely used measure of the variability or dispersion.

It shows how much variation there is from the **"Average" or Mean**.

It is denoted by the **letter σ**.

For **Small samples, n<30**

$$SD = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}}$$

For **Large samples, n>30**

$$SD = \sqrt{\frac{\sum (X - \bar{X})^2}{n}}$$

3. Unpaired or Independent 'Student's' t-test

- The unpaired, or "independent samples" *t*-test is used when two separate independent and identically distributed samples are obtained, one from each of the two populations being compared.

$$\mathbf{t = \frac{\bar{X}_1 - \bar{X}_2}{SE\ (\bar{x}_1 - \bar{x}_2)}}$$

Where,

- $\overline{X_1}$ = Mean of the first group
- $\overline{X_2}$ = Mean of the second group
- **SE ($\bar{x}_1 - \bar{x}_2$) = Standard Error of difference between the 2 means**

- The **degrees of freedom** used in this test is $n_1 + n_2 - 2$.

4. Paired or Dependent 'Student's' t-test

- **The paired t-test** provides a hypothesis test of the difference between population means for a pair of random samples whose differences are approximately normally distributed.
- **The test statistic is calculated as:**

$$t = \frac{\bar{d}}{\sqrt{s^2 / n}}$$

Where is the mean difference,

s^2 is the sample variance

n is the sample size.

t is a Student t quantile with

n-1 degrees of freedom.

5. Level of Significance (p-value)

- The maximum probability of rejecting a correct null hypothesis.
- In testing a given hypothesis, the maximum probability with which we would be willing to take risk is called Level of Significance of the Test.

- **P-value ≥ 0.05 – non-significant.**
- **P-value < 0.05 – significant.**
- **P-value < 0.01 - highly significant.**
- **P-value < 0.001 - very highly significant.**

RESULTS

RESULTS

In this two arm clinico-biochemical study, the total number of patients enrolled (72) were divided into two groups: Control and test group which were treated with NSPT alone and with phase-I therapy along with pocket irrigated with water containing ozone. The parameters: PPD, CAL, PI, GI and IL-1β1 level were assessed.

CLINICAL MEASUREMENTS:

PROBING POCKET DEPTH

INTERGROUP COMPARISONS

The differences in mean value of PPD of **Control group (4.20± 0.401)** and **Test group (4.19± 0.624)** at **baseline** was found to be **statistically insignificant (p>0.05).** Post **1 month,** the difference in mean PPD of **Control (2.14± 0.351)** and **Test group (2.11± 0.319)** was found to be **statistically insignificant (p>0.05).** Furthermore, at **3months,** the difference in mean value of PPD of **Control group (2.12±0.447)** and **Test group (2.05± 0.609)** was found to be **significant statistically (p<0.05).** ***(Table 1, Graph 1)***

INTRAGROUP COMPARISONS

Control group (SRP alone) demonstrated a reduction which was **significant statistically**. The mean PPD of **4.20 ± 0.401 at baseline** showed significant reduction of **2.06 ± 0.249** at **one month (p < 0.05),** indicating notable improvement in PPD. Similarly, PPD further decreased by **2.08 ± 0.169** at **three months, (p < 0.05)** with a difference which was **significant statistically** from baseline. ***(Table 1)***

Test group (SRP + ozonated water) demonstrated a statistically significant reduction in PPD scores over time. The **baseline** mean PPD of **4.19 ± 0.624 showed significant reduction of**

2.00 ± 0.539 at **one month (p < 0.05)**, demonstrating a **statistically significant** improvement in periodontal health. Similarly, PPD further decreased by **2.14 ± 0.497** at **three months(p<0.05)**, with a **significant** difference from baseline. ***(Table 1)***

CLINICAL ATTACHMENT LEVEL

INTERGROUP COMPARISON

Difference in mean of CAL in **Control group (4.19± 0.632)** and **Test group (4.44 ± 0.689)** at **baseline** was found to be **statistically non-significant (p>0.05).** At **1 month,** the difference in mean value of clinical attachment level of **Control group (3.99± 0.451)** and **Test group (3.69 ± 0.657)** was **statistically insignificant (p>0.05).** Post **3 months,** mean of CAL in **Control group (2.56± 0.348)** and **Test group (1.36 ± 0.289)** was found to be **significant statistically (p<0.05).** ***(Table 2, Graph 2)***

INTRAGROUP COMPARISON

Control group (SRP alone) demonstrated a reduction which was significant statistically over time. The **baseline** mean CAL of **4.19± 0.632** decreased by **0.2 ± 0.602** at **one month (p >0.05),** indicating statistically insignificant difference from baseline Similarly, CAL decreased by **1.63±0.253 at three months(p<0.05),** being **statistically significant** from baseline. ***(Table2)***

Test group (SRP + ozonated water) demonstrated reduction which was significant statistically over time. The **baseline** mean of CAL of **4.44 ± 0.689 significantly** decreased by **0.75 ± 0.572** at **one month (p = 0.05**), indicating an initial gain in attachment. Similarly, CAL decreased by **3.08 ± 0.478 (p < 0.05),** demonstrating a difference which was **significant statistically** from baseline. ***(Table 2)***

BIOCHEMICAL PARAMETER

SALIVARY INTERLEUKIN 1-BETA

INTERGROUP COMPARISON

The differences in mean value of salivary interleukin 1-beta level in **Control group (13.76± 1.65)** and **Test group (14.05± 1.13)** at **baseline** was **insignificant statistically (p>0.05).** At **3 months,** the difference in mean value of salivary interleukin 1-beta level in **Control group (12.50±1.28)** and **Test group (11.89± 1.18)** was **statistically significant (p<0.05).** ***(Table 3, Graph 3)***

INTRAGROUP COMPARISON

IL-1 Beta (IL-1β) in **control group** (SRP alone) revealed a **significant** reduction over three months. The **baseline** IL-1 β level of **13.76± 1.65** decreased by **1.26±1.02** at **three months(p<0.05),** demonstrating a **significant** decrease in inflammatory cytokine level following treatment. ***(Table 3)***

IL-1 Beta (IL-1β) in the **test group** (SRP + ozonated water) demonstrated a **significant** reduction over **three months**. The **baseline** IL-1β level of **14.05 ± 1.13** significantly decreased by **2.16 ± 1.39** at **three months (p < 0.05),** resulting in a **statistically significant** decrease in inflammatory cytokine levels following treatment. ***(Table 3)***

PLAQUE INDEX

INTERGROUP COMPARISON

The differences in mean value of PI of **Control group (2.83± 0.507)** and **Test group (2.86±**

0.424) at **baseline** was **statistically insignificant (p>0.05).** The difference in **1 month** mean value of PI of **Control group (1.72± 0.566)** and **Test group (1.47± 0.609)** was **statistically insignificant (p>0.05).** The **3 months** difference in PI (mean) of **Control group (1.70±0.591)** and **Test group (1.08± 0.280)** was **significant statistically (p<0.05).** ***(Table 4, Graph 4)***

INTRAGROUP COMPARISON

Control group (SRP alone) demonstrated a reduction in PI which was **significant statistically** over time. Mean PI of **2.83 ± 0.507** at **baseline** decreased by **1.11 ± 0.471** at **one month (p < 0.05),** indicating a significant improvement in plaque index. Similarly, the PI score further decreased by **1.13 ±0.381** at **three months(p<0.05)** with a significant difference from baseline. ***(Table 4)***

Test group (SRP + ozonated water) demonstrated a statistically significant reduction in Plaque Index (PI) scores over time. The **baseline** mean PI score of **2.86 ± 0.424 s**ignificantly decreased by **1.39 ± 0.389** at **one month (p < 0.05),** indicating a notable improvement in plaque control. Similarly, the PI score significantly decreased by **1.78 ± 0.284 at three months (p < 0.05)** from baseline ***(Table 4)***

GINGIVAL INDEX

INTERGROUP COMPARISON

Differences in mean GI of **Control group (2.99±0.399)** and **Test group (2.89± 0.398)** at **baseline** was **statistically insignificant (p>0.05).** The **1-month** difference in GI (mean) of **Control group (1.81± 0.467)** and **Test group (1.61± 0.645)** was **statistically insignificant (p >0.05).** Furthermore the **3 months** difference in mean GI in **Control group (1.64±0.683)** and **Test group (1.53± 0.654)** was **statistically insignificant (p>0.05).** ***(Table 5, Graph 5)***

INTRAGROUP COMPARISON

Control group demonstrated a **statistically significant** reduction in GI scores over time. The mean GI score of **2.99 ± 0.399(baseline)** significantly reduced by **1.18 ± 0.117** at **one month ($p < 0.05$),** demonstrating a significant improvement in gingival index. Similarly, the GI score further decreased **statistically** by **1.35 ± 0.049** at **three months**, from baseline **($p < 0.05$)**. ***(Table 5)***

Test group (SRP + ozonated water) demonstrated a reduction which was **significant statistically**. Mean GI score of **2.89 ± 0.398(baseline)** decreased significantly by **1.28 ± 0.285** at **one month ($p < 0.05$),** indicating a notable improvement in gingival index. Similarly, the GI score further decreased by **1.36 ± 0.169** at **three months**, being **significant statistically** from baseline **($p < 0.05$).** ***(Table 5)***

TABLES AND GRAPHS

Table 1: Intergroup & Intragroup comparison of Probing Pocket Depth (PPD) (in mm) of control and test group over a period of time

Inter-group comparison

	CONTROL GROUP		**TEST GROUP**		p-value
Probing Pocket Depth	Mean	Std. Deviation	Mean	Std. Deviation	
Baseline	4.20	.401	4.19	.624	1.000
1 month	2.14	.351	2.11	.319	0.726
3 months	2.12	.447	2.05	.609	**0.008****

Intra-group comparison

CONTROL GROUP				**TEST GROUP**			
Probing Pocket Depth	Mean	Std.Deviation	p-value		Mean	Std. Deviation	p-value
Baseline	4.20	.401	-	Baseline	4.19	.624	-
Baseline vs 1 month	2.06	0.249	**0.03***	Baseline vs 1 month	2.00	0.539	**0.02***
Baseline vs 3 month	2.08	0.169	**0.002****	Baseline vs 3 month	2.14	0.497	**0.001****

***P<0.05 significant**

Graph 1: Intergroup comparison of Probing Pocket Depth (PPD) (in mm) of control and test group over a period of time

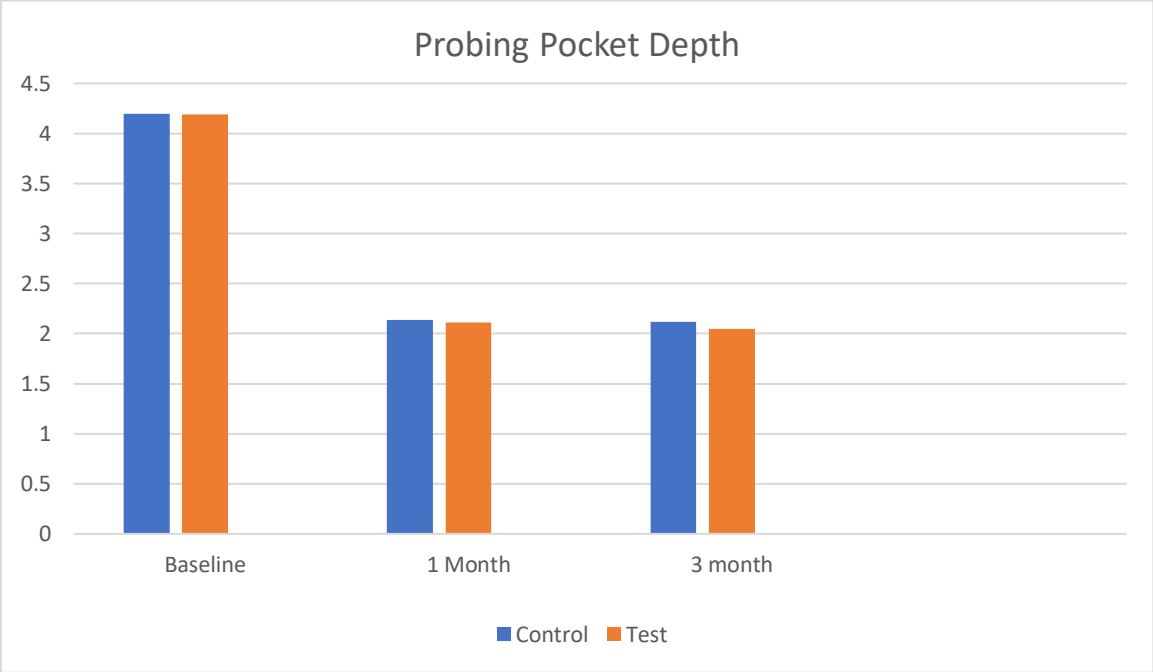


Table 2: Intergroup & Intragroup comparison of Clinical Attachment Level (CAL) (in mm) of control and test group over a period of time

Intergroup comparison

	CONTROL GROUP		**TEST GROUP**		p-value
Clinical Attachment Level	Mean	Std. Deviation	Mean	Std. Deviation	
Baseline	4.19	0.632	4.44	0.689	0.443
1 month	3.99	0.451	3.69	0.657	0.696
3 months	2.56	0.348	1.36	0.289	**0.001****

Intra-group comparison

CONTROL GROUP				**TEST GROUP**			
Clinical Attachment Level	Mean	Std.Deviation	p-value		Mean	Std. Deviation	p-value
Baseline	4.19	0.632	-	Baseline	4.44	0.689	-
Baseline vs 1 month	0.2	0.602	0.06	Baseline vs 1 month	0.75	0.572	**0.05***
Baseline vs 3 month	1.63	0.253	**0.001****	Baseline vs 3 month	3.08	0.478	**0.002****

***P<0.05 significant**

Graph 2: Intergroup comparison of Clinical Attachment Level (CAL) (in mm) of control and test group over a period of time

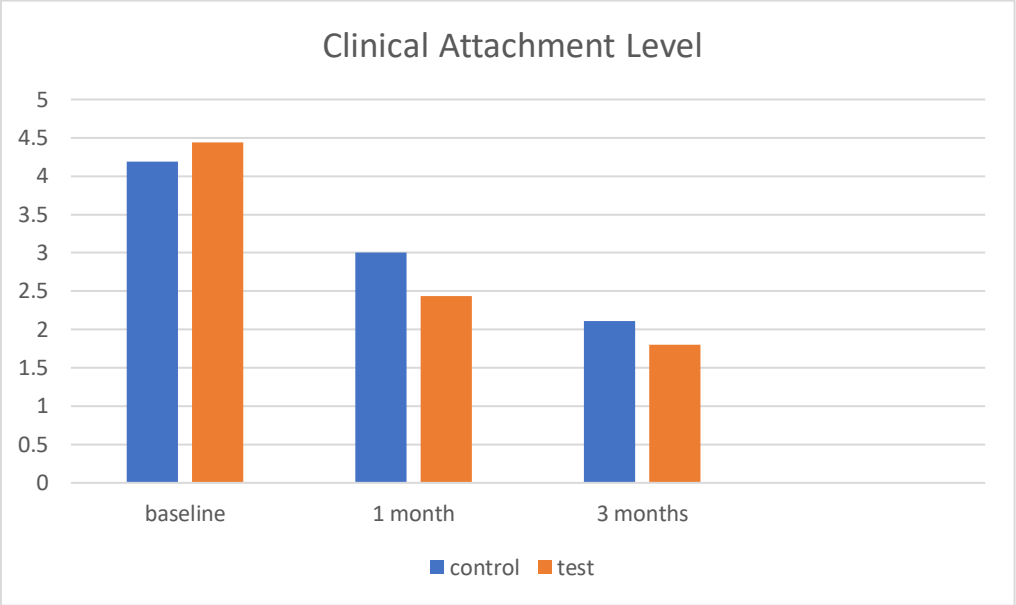


Table 3: Intergroup & Intragroup comparison of Salivary Interleukin 1 -beta (IL-1β (in pg/ml) of control and test group over a period of time

Intergroup Comparison					
	CONTROL GROUP		**TEST GROUP**		p-value
Salivary interleukin 1 beta	Mean	Std. Deviation	Mean	Std. Deviation	
Baseline	13.76	1.65	14.05	1.13	0.398
3 months	12.50	1.28	11.89	1.18	**0.03***

Intra-group comparison							
CONTROL GROUP				**TEST GROUP**			
Salivary interleukin 1 beta	Mean	Std.Deviation	p-value		Mean	Std. Deviation	p-value
Baseline	13.76	1.65	-	Baseline	14.05	1.13	-
Baseline vs 3 month	1.26	1.02	**0.02***	Baseline vs 3 month	2.16	1.39	**0.001****

***P<0.05 significant**

Graph 3: Intergroup comparison of Salivary Interleukin 1-beta (IL-1β) (in pg/ml) of control and test group over a period of time

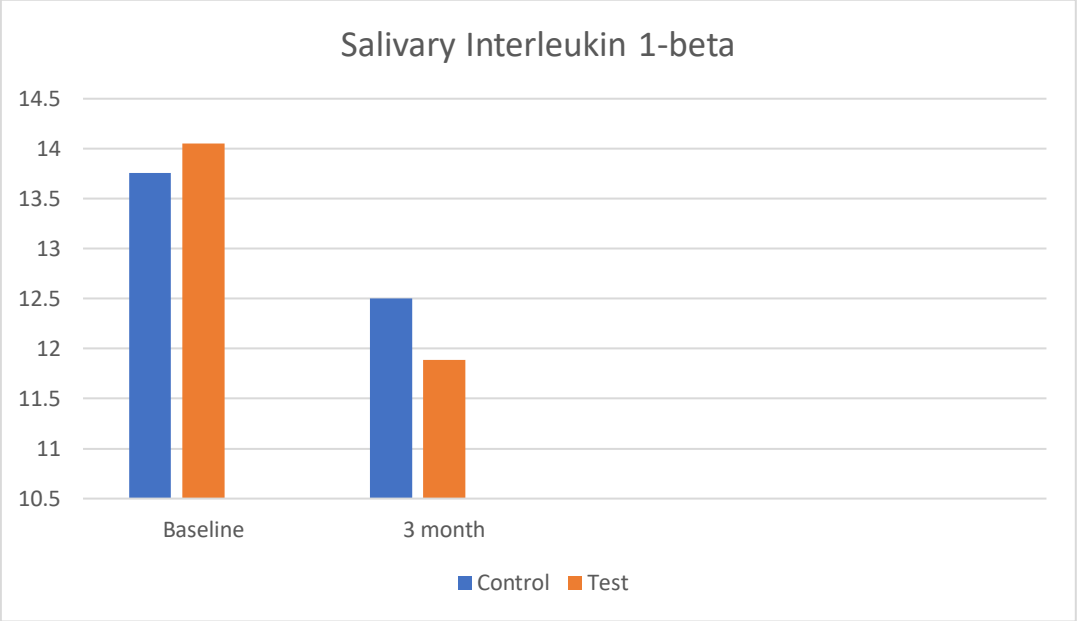


Table 4: Intergroup & Intragroup comparison of Plaque Index (PI) (in mm) of control and test group over a period of time

Intergroup Comparison

	CONTROL GROUP		**TEST GROUP**		p-value
Plaque Index	Mean	Std. Deviation	Mean	Std. Deviation	
Baseline	2.83	.507	2.86	.424	0.802
1 month	1.72	.566	1.47	.609	0.076
3 months	1.70	.591	1.08	.280	**0.002****

Intra-group comparison

CONTROL GROUP				**TEST GROUP**			
Plaque index	Mean	Std.Deviation	p-value		Mean	Std. Deviation	p-value
Baseline	2.83	0.507	-	Baseline	2.86	0.424	-
Baseline vs 1 month	1.11	0.471	**0.04***	Baseline vs 1 month	1.39	0.389	**0.03***
Baseline vs 3 month	1.13	0.381	**0.02***	Baseline vs 3 month	1.78	0.284	**0.01***

***P<0.05 significant**

Graph 4: Intergroup comparison of Plaque Index (PI) (in mm) of control and test group over a period of time

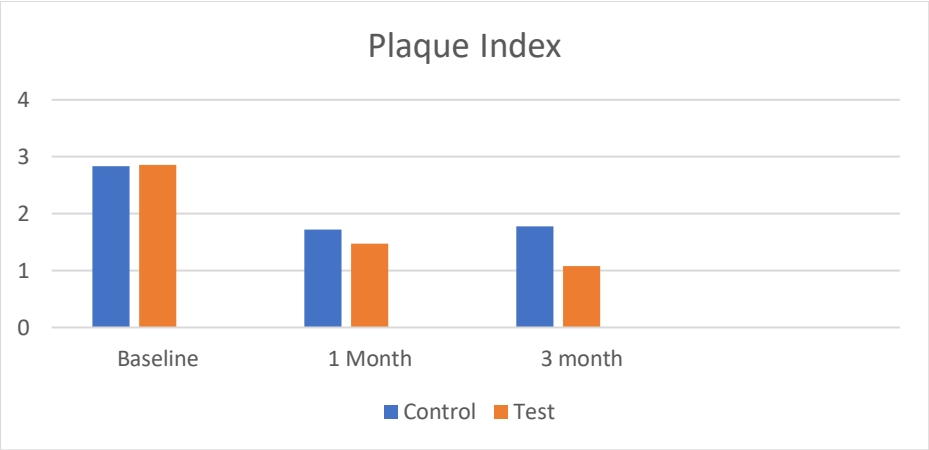


Table 5: Intergroup& Intragroup comparison of Gingival Index (GI) (in mm) of control and test group over a period of time

Intergroup Comparison

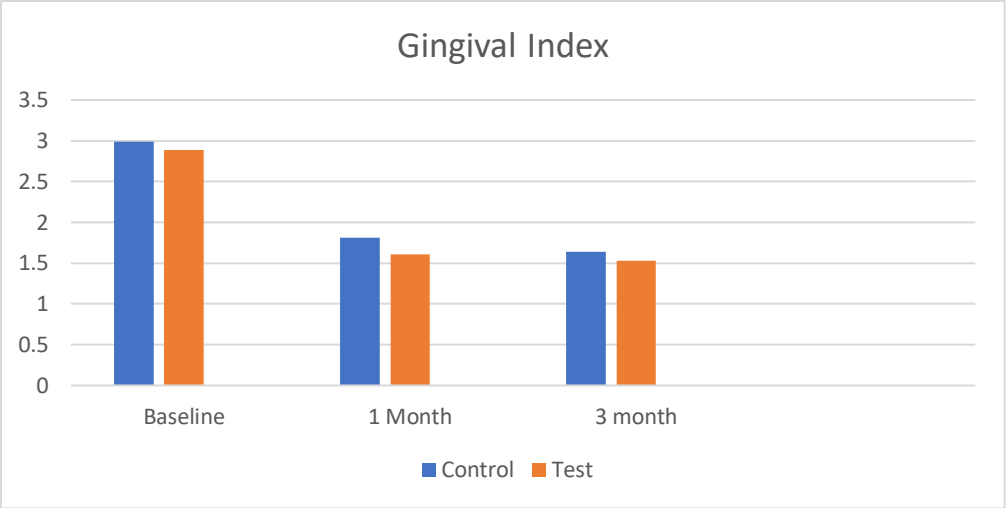
	CONTROL GROUP		**TEST GROUP**		p-value
Gingival Index	Mean	Std. Deviation	Mean	Std. Deviation	
Baseline	2.99	0.399	2.89	.398	1.000
1 month	1.81	0.467	1.61	.645	0.147
3 months	1.64	0.683	1.53	.654	0.483

Intra-group comparison

CONTROL GROUP				**TEST GROUP**			
Gingival index	Mean	Std.Deviation	p-value	Gingival index	Mean	Std. Deviation	p-value
Baseline	2.99	0.399	-	Baseline	2.89	0.398	-
Baseline vs 1 month	1.18	0.117	**0.03***	Baseline vs 1 month	1.28	0.285	**0.02***
Baseline vs 3 month	1.35	0.049	**0.01***	Baseline vs 3 month	1.36	0.169	**0.004***

***P<0.05 significant**

Graph 5: Intergroup comparison of Gingival Index (GI) (in mm) of control and test group over a period of time



DISCUSSION

DISCUSSION

Based on clinical and biochemical parameters, the RCT was set out to assess the potency of ozone water as a supplement to scaling and root planing (SRP). 72 participants were randomly allocated (by coin toss technique) to one of the two groups according to the given inclusion criteria: either SRP (GROUP A) or ozone water irrigation along with SRP (GROUP B). In this study, the effects of ozone water irrigation were assessed using a one-month and three-month post scaling. All clinical and biochemical parameters were measured at baseline 1 and 3 months.

A key clinical metric for evaluating periodontal disease and the efficacy of treatment measures is probing pocket depth (PPD). PPD at baseline (control: 4.20 ± 0.401mm; test: 4.19 ± 0.624mm) and at one month (control: 2.14 ± 0.351mm; test: 2.11 ± 0.319mm) did not differ significantly between groups, according to intergroup comparisons, indicating that both groups had similar baseline circumstances and early healing responses. At the 3-month point, however, a statistically significant difference ($p < 0.05$) had been seen between the test (2.05 ± 0.609mm) and the control group (2.12 ± 0.447mm). Ozone's oxidative activity, which breaks down bacterial biofilms, may be responsible for the test group's further improvement in PPD. Its stimulatory effects on local circulation and oxygenation, on the other hand, may help increase tissue response and stabilize pocket reduction. These findings are in line with those of *Musalaiah et al.*[29], *Ramzy et al.*[46], *and Nagayoshi et al.*[17], which quoted, employing ozone water as a supplement to oral prophylaxis in the treatment of chronic periodontitis significantly decreases probing pocket depth both within and across groups. These results are in contrast with the results of *Habashneh et al*[41], who showed a non- significant difference in PPD between control and test group as according to them the use of distilled water in control group could have attributed to the non-significant results between the two groups.

Intragroup comparison revealed progressive improvement in probing pocket depth within both the groups under study. In control group slight reduction was observed between baseline to one month {2.06 ± 0.249mm(p=0.03)} and three months {2.08 ± 0.169mm(p=0.002)} demonstrating a significant reduction in pocket depth that persisted over a period of three months. The test group demonstrated more pronounced reduction in PPD between baseline to one month {2.00±0.539mm(p=0.02)} and three month {2.14± 0.497mm(p=0.001)}, demonstrating a significant reduction. *Kshitish & Laxman*[26] reported a similar result in both

intergroup and intragroup analysis following the use of ozonated water. These intragroup findings support the clinical effectiveness of both treatments in lowering periodontal pocket depths and promoting periodontal healing.

Clinical Attachment Level (CAL), which reflects severity of disease, effectiveness of treatment, is a crucial metric for evaluating the results of periodontal therapy. Intergroup differences were insignificant ($p > 0.05$) at baseline (control: 4.19 ± 0.632mm; test: 4.44 ± 0.689mm) and at one month (control: 3.99 ± 0.451mm; test: 3.69 ± 0.657mm), suggesting similar starting circumstances and early treatment responses. But later on, the CAL at 3 months (1.36 ± 0.289mm) for test group and control group (2.56 ± 0.348mm) was statistically detectable, indicating that using ozonated water as an adjuvant had a higher long-term advantage in clinical attachment gain. Ozone's antibacterial, improved local oxygenation, and healing-promoting qualities may be responsible for the test group's greater CAL improvement. In a study to determine the clinical effectiveness of ozone water, *Nagayoshi et al*[17] *and Huth et al*[20] also found similar findings, reporting a substantial decrease in clinical attachment level both within and across groups. Contrasting results are shown by *Habashneh et al.*[41], which demonstrated a non-significant reduction in CAL between the groups.

In intragroup comparison, both group A and group B showed significant difference in CAL from baseline to 1 month {0.2± 0.602mm (p=0.06)},{0.75 ± 0.572mm (p =0.05)} and 3 months {1.63 ± 0.253 mm(p=0.001)},{3.08 ± 0.478mm (p=0.002)}.These results demonstrate the efficacy of SRP along with ozone water in controlling the loss of periodontal tissue by showing that both treatment methods resulted in a notable increase in clinical attachment level. The above observations are in accordance with prospective split-mouth research of *Musailah*[29] *and Alsakr et al.*[30] who evaluated the effectiveness of ozone therapy in treating stage II and stage III periodontitis. The study found that all clinical variables significantly decreased both within and between groups from baseline to six weeks.

The etiology and development of periodontal disease are tightly linked to cytokine that promote inflammation like interleukin-1 beta (IL-1β). Saliva with elevated IL-1β levels is a useful biomarker for assessing the host's reaction to periodontal treatment since it indicates active inflammation and tissue degradation. This conclusion is further supported by the intergroup comparison. At three months, a significant difference (control: 12.50 ± 1.28mm vs. test: 11.89 ± 1.18mm; $p < 0.05$) appeared favoring the test group, even though the groups' baseline IL-1β

levels were statistically identical (control: 13.76 ± 1.65mm; test: 14.05 ± 1.13mm; p > 0.05). This implies that, in comparison to SRP alone, supplementary ozonated water treatment may provide improved control of the inflammatory response. The above findings are in accordance with those of *Katti & Chava*[43] who found that, as compared to oral prophylaxis alone (control group), there was a substantial intergroup and intragroup decrease in the interleukin 1-beta level with ozone water (test group).

Intragroup comparison revealed a progressive improvement in IL-1β level within both the groups under the study. In the control group slight reduction was observed in IL-1β between baseline to three months {1.26 ± 1.02mm(p=0.02)}. In test group more pronounced reduction was observed in IL-1β level from baseline to 3 months {2.16± 1.39 mm (p=0.001)}. The significant reduction not only validates the anti-inflammatory effect of SRP but also emphasizes the possible supplementary advantage of ozonated water. Because of its well-established antibacterial and anti-inflammatory qualities, ozone may help better regulate the host immune response, which would improve periodontal repair and lower levels of inflammatory mediators. These, observations are similar with those of *Uraz et al.*[38] who demonstrated a significant decrease in IL-1β both within and between groups following the additional use of ozone water.

Silness and Löe[49] created the plaque index (PI), that gauges the plaque thickness at the gingival edge. Comparable beginning points and early plaque control outcomes were indicated by intergroup comparisons, which showed that the difference in mean PI scores between groups was not statistically significant at baseline (control: 2.83 ± 0.507mm, test: 2.86 ± 0.424mm; p > 0.05) or at one month (control: 1.72 ± 0.566mm, test: 1.47 ± 0.609mm; p > 0.05). By three months, however, the test group's PI score (1.08 ± 0.280) was statistically lower to control group (1.70 ± 0.591mm) (p < 0.05). This implies that when combined with traditional SRP, the supplementary use of ozonated water irrigation produced a better plaque-reducing impact over time. The above results were in accordance with *Aggarwal P*[28] *and Ranjith et al.*[31] who conducted a clinic-microbiological study and showed a significant reduction both within and across groups. The results of this study are in contrast with the study conducted by *Habashneh et al.*[41] which showed a non-significant reduction in PI on intergroup comparison.

The intragroup comparison of both group A and group B showed significantly relevant decline in PI from baseline to 1 month {1.11± 0.471mm (p=0.04)}, {1.39± 0.389mm, p=0.03)}and 3

months {1.13±0.381mm (p=0.02)},{1.78 ± 0.284mm, p=0.01)}.These findings support SRP's ability to lower plaque levels and enhance oral hygiene over time when used with ozone water. These results are in accordance with the results of *Musalaiah et al.*[29] who compared the effectiveness of ozonated water with NSPT alone in treating chronic periodontitis. They demonstrated that subgingival irrigation of ozonated water with SRP significantly reduced the plaque index both within and between groups.

Löe and Silness [50] developed the Gingival Index (GI), a clinical measure that evaluates the degree of gingival inflammation by looking at color, consistency, and bleeding on probing. However, no statistically significant changes were found at any given point of time when comparing between the groups. Similar initial gingival circumstances were confirmed by the comparable GI scores of the test (2.89 ± 0.398mm) and control (2.99 ± 0.399mm) at baseline ($p > 0.05$). All intergroup results were insignificant ($p > 0.05$), with control group gingival index at 1 month being 1.81 ± 0.467mm, the test group's being 1.61 ± 0.645mm, and after 3 months being 1.64 ± 0.683mm(control) and 1.53 ± 0.654mm(test). The short follow-up period, sample size restrictions, or the ozone dosage and administration mechanism may be responsible for the lack of significant intergroup differences. Thus, SRP by itself considerably lowers gingival inflammation but in this study ozone water irrigation as an adjuvant did not produce statistically better results. Similar observations are demonstrated by *Habashneh et al.*[41] which found that using ozone water as a supplement to NSPT in chronic periodontitis resulted in non-significant intergroup difference.

Both control and test group showed significant decline in GI from baseline to 1 month {1.18 ± 0.117mm (p=0.03}), {1.28 ± 0.285 mm (p=0.02)}and 3 months {1.35 ± 0.049mm (p=0.01)}, {1.36 ± 0.169mm(p=0.004}). These results support SRP's well-established use as a primary periodontal treatment by showing how well it reduces gingival inflammation. Concurrent results were demonstrated by *Nagayoshi et al.*[17], who found that using ozone water in addition to SRP significantly decreased gingival index within a group.

This study demonstrated that ozone therapy improves healing results by lowering levels of interleukin1-beta, a pro-inflammatory cytokine in periodontal disease. Future studies with larger number of participants, prolonged follow-up are necessary to authenticate these findings and investigate underlying processes and protracted advantages of ozone therapy in managing periodontal conditions. By offering a more accurate and impartial comprehension of the clinical

criteria, the use of biochemical indicators significantly enhanced the assessment.

According to this study, ozone water irrigation is more beneficial than phase I therapy alone in managing CP when used in conjunction with SRP.

SUMMARY

SUMMARY

The present study evaluated and compared the efficacy of two treatment modalities in the management of CP. SRP alone and SRP combined with subgingival irrigation using ozonated water. Both treatment approaches independently demonstrated clinical benefits, indicating their effectiveness in improving periodontal health. However, the comparative analysis between the SRP alone and SRP with ozonated water revealed that the adjunctive use of ozonated water significantly enhanced clinical outcomes. The superior clinical and biochemical outcome observed in the test group can be attributed to the therapeutic properties of ozonated water. Ozone is known for its antimicrobial, anti-inflammatory, and immunomodulatory effects. Its application in subgingival irrigation likely enhanced the decontamination of periodontal pockets and contributed to the modulation of the host inflammatory response, thereby facilitating improved periodontal healing.

Summarizing the results of our study-

- Probing pocket depth (PPD) demonstrated significant reductions both within each group and between groups, with the test group exhibiting more pronounced improvement from baseline to 1 and 3 months, indicating the added benefit of the adjunctive therapy.
- Clinical attachment level (CAL) demonstrated marked improvement within and between both the groups, with the test group exhibiting superior gains at 1and 3 months follow-up from baseline, underscoring the efficacy of the adjunctive therapy.
- From baseline to 3 months, interleukin-1 beta (IL-1β) levels significantly dropped both within group over time and between groups, with the test group experiencing a more pronounced decrease, indicating the superior anti-inflammatory benefits of the adjunctive treatment.

- Both intergroup and intragroup comparison demonstrated significant reduction in plaque index (PI) at 1 month and 3 months follow up period.
- There was insignificant difference in gingival index (GI) in between the groups, however, both groups demonstrated a significant decrease in GI from baseline through 1 month and 3 months.

However, future research with larger sample size, longer follow up periods are warranted to validate these results and explore the long-term benefits and underlying mechanisms of ozone therapy in periodontal treatment.

CONCLUSION

CONCLUSION

Acknowledging the limitations of this study, it may be concluded that ozonated water plays an additional role when used along with SRP in deep periodontal pockets. Adjunctive use of ozonated water as an adjunct to SRP for better clinical and biochemical effects may be recommended.

In conclusion, while SRP remains a cornerstone of periodontal therapy, the adjunctive use of ozonated water provides additional clinical and biochemical benefits. This combination therapy holds promise as an effective strategy for enhanced treatment of chronic periodontitis.

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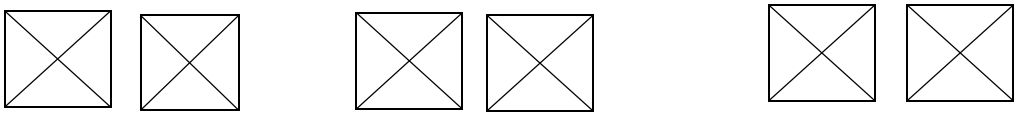
ANNEXURES

ANNEXURE-1

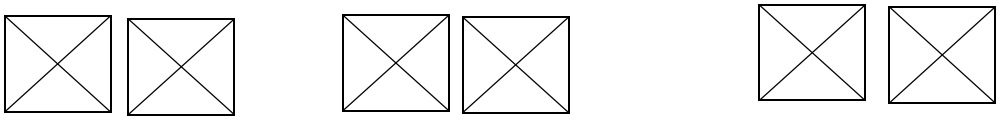
THESIS CASE HISTORY
DEPARTMENT OF PERIODONTICS AND ORAL IMPLANTOLOGY
SANTOSH DENTAL COLLEGE, GHAZIABAD

Name of the Investigator – Dr. Tanya Sahai
TOPIC - Evaluation of the efficacy of the ozonated water as an adjunct to scaling and root planing in the management of chronic periodontitis.
Type of Group: TEST..…….. CONTROL……..

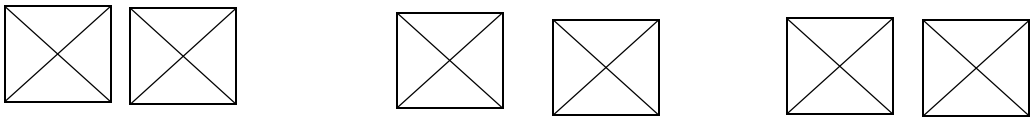
NAME OF THE PATIENT:
AGE/SEX:
OPD NO.:
DATE:
OCCUPATION:
CHIEF COMPLAINT:
DIAGNOSIS:
CLINICAL PARAMETERS
AT BASELINE: 1 MONTH 3MONTHS
Probing Pocket Depth



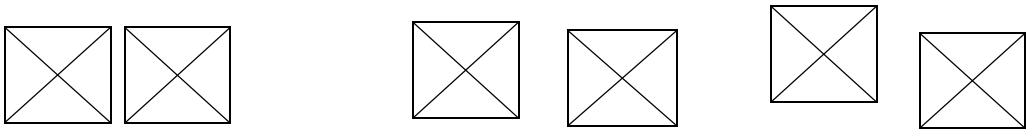
Clinical Attachment Level Gain



Plaque Index (Loe and Silness)



Gingival Index (Loe and Silness)



BIOCHEMICAL ANALYSIS: ELISA TEST
Inflammatory marker level – INTERLEUKIN 1-BETA (IL-1β)
AT BASELINE: 3 MONTHS

ANNEXURE-2

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

Department of Periodontics and oral implantology

CONSENT FORM

1. I _____, age__ son/daughter of R/O_gives full consent for the examination, investigation and the treatment, without any compulsion of any kind.

2. Doctor has fully explained to me the purpose of the procedure(s), the predictable and unpredictable consequences of the procedure, drug administered if any including the use of local anaesthesia, and has also informed me of expected benefits and complications (from known and unknown causes), discomforts and risks that may arise as well as possible alternatives to the proposed treatment including no treatment. The risks of no treatment have also been discussed. I have been given an opportunity to ask questions and all my questions have been answered fully and satisfactorily.

3. I understand that during the course of the procedure(s), unforeseen conditions may arise which necessitates procedures different from those contemplated. I, therefore, consent to the performance of additional procedures that the above-named dentist or his/her associate may consider necessary.

4. I acknowledge that no guarantee or assurances have been made to me concerning the results intended from the procedure(s).

5. No identity lies with the doctor in case the treatment is discontinued or the outcome is not as predicted.

6. I confirm that I have read and fully understand the above and that all blank spaces have been completed prior to my signing. I give my consent that this record can be used for research, education, training & upliftment of the dental profession.

Signature of patient Date:

HEAD OF DEPARTMENT

ANNEXURE-3

सन्तोष डेन्टल कॉलेज

प्रताप विहार, गाजियाबाद

सहमति पत्र

1. मैं .....पुत्र/पत्री.....निवासी..... ..... अपने ऊपर किये जाने वाले निरीक्षण/ परीक्षण, चिकित्सीय सलाह/पद्धति की पूर्ण सहमति देता/देती हुँ।

2. मुझे चिकित्सा प्रणाली और चिकित्सा के उपरान्त होने वाले संभावित नतीजों के बारे में दंत चिकित्सक के द्वारा अच्छी तरह समझाया गया है।

3. मुझे चिकित्सा के दौरान अथवा उसके उपरांत होने वाली संभावित/ अप्रत्याशित हानियों, इलाज के समय उपयुक्त होने वाली दवा व उसके प्रयोग के बारे में भी अच्छी तरह बताया गया है।

4. रोगी के द्वारा चिकित्सा के बीच में ही छोड़कर जाने अथवा चिकित्सा के उपरान्त परिणाम के उम्मीद के अनुसार न आने पर, चिकित्सक की जिम्मेदारी नहीं होगी।

5. मैं पुनः अपनी सहमति देता हूँ कि इस रिकार्ड को भविष्य में चिकित्सीय शोध, प्रशिक्षण एवं चिकित्सा प्रणाली के उत्थान के लिये प्रयोग किया जा सकता है।

तारीखः

रोगी के हस्ताक्षर

ANNEXURE-4

SANTOSH
Deemed to be University
(Established u/s 3 of the UGC Act, 1956)

F. No. SU/2023/2480[14] Dated: 26.09.2023

Subject: Grant of Ethical Clearance for Thesis Protocol Topic "Evaluation of the efficacy of ozonated water as an adjunct to scaling and root planing in management of chronic periodontitis: A randomized control study", submitted by **Dr. TANYA SAHAI** ,MDS student of Batch 2022-2023, Department of **PERIODONTOLOGY**.

*****

With reference to his/her request for grant of Ethical Clearance for the Thesis Protocol entitled "Evaluation of the efficacy of ozonated water as an adjunct to scaling and root planing in management of chronic periodontitis: A randomized control study", **Dr. TANYA SAHAI** is informed that the Thesis Protocol submitted by him/her was considered by the **Screening Committee** of the Santosh Dental College & Hospital in its meetings held on 07.02.2023 & 08.02.2023. The recommendations of the **Screening Committee** were considered in detail by the Institutional **Ethics Committee** in its meeting held on **23.09.2023** and the same was **approved** by the **Ethics Committee**.

He /She is informed accordingly for further necessary action.

[DR. NARESH SHARMA]
REGISTRAR

SANTOSH "DEEMED TO BE UNIVERSITY" REGISTRAR GHAZIABAD, NCR DELHI

Dr. TANYA SAHAI
MDS student of batch 2022-2023
Department of PERIODONTOLOGY
Email: tanyasahai96703@gmail.com

Copy to:
1. The Vice Chancellor
2. The Dean, SDC&H
3. The Dean – Research
4. The Dean – Academics
5. The Director - IQAC
6. **Dr. Shweta Bali** (Guide)
7. HOD of **PERIODONTOLOGY**

ANNEXURE-5

Tanya Sahai
Evaluation of the efficacy of ozonated water as an adjunct to scaling and root planing in the managem

Santosh University, Ghaziabad

Document Details

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